REMARKS

Applicants request reconsideration of this application in view of the foregoing amendments and following remarks.

I. Restriction/Election Requirement

The Office action made the prior restriction/election requirement final, and claims 16-24 are considered withdrawn from consideration in the present application. While applicants disagree that these claims should be restricted from consideration in the present application, applicants nevertheless are canceling claims 16-24 from this application without prejudice. Applicants reserve the right to file continuing applications directed to the features of claims 16-24.

II. Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 1-15 and 25-27 are rejected as allegedly being indefinite. Applicants traverse the rejection of these claims. Each of the rejections cited in the Office action is addressed below in detail.

Applicants respectfully disagree with the statements in the Office action that (1) no method step is affirmatively recited that allows detection of a bioactive compound or organism, and (2) detecting a change is not sufficient to accomplish the claimed method. Exposing chromatophores to a bioactive compound or organism induces an observable optical change in the chromatophores. Absent such exposure, the optical characteristics of the chromatophores are substantially static, i.e., there is no readily observable optical change in the chromatophores. Thus, if an optical change occurs, it is a direct indication that the chromatophores were exposed to a bioactive compound or organism.

Applicants have amended claim 1 to recite detecting an optical change. This amendment addresses the Examiner's query as to what type of change is required to positively detect a bioactive compound or organism.

Applicants have amended claim 1 to refer to both a bioactive compound and an organism. While the term "bioactive compound" as used in the application was intended to include organisms (see page 8, lines 1-2, for example), applicants nonetheless have amended

claim 1 to address the alleged indefiniteness resulting from applicants' use of "bioactive compound" in the claims. Applicants also have amended claim 5 to specifically refer to "organism" to address the rejection under 35 U.S.C. § 112, second paragraph.

Applicants have amended claim 7 to address the objection to the preamble and antecedent basis for the phrase "The method of identifying".

Applicants have amended claim 7 to recite exposing a first type of chromatophore to a sample potentially comprising a bioactive compound or organism, and exposing a second type of chromatophore to a sample potentially comprising a bioactive compound or organism. The purpose of the present invention is to be able to detect a bioactive compound or organism in any sample that is capable of supporting either the compound or organism in an active state. Applicants assert that this purpose is clear from the claim language, particularly in view of the considerable detail concerning these features provided by the specification. Nevertheless, applicants have amended claim 7 as suggested by the Examiner, and hence request that this rejection be withdrawn.

Applicants disagree that the type of response required to detect the bioactive compound or organism is not clear from the claim language in view of the specification. The present application provides considerable detail concerning optical changes that are indicative of the presence of the bioactive compound or organism, including many figures which depict the optical changes that occur in the presence of the bioactive compound or organism. Footnote number one summarizes drawings and photographs that the present application provides to exemplify the optical response that occurs when chromatophores are exposed to bioactive compounds or organisms according to the present invention.[1] This information alone provides

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[1] For example, FIGS. 1A-1B illustrate pigment aggregation in chromatophores obtained from fish of the genus Betta prior to and after exposure to norepinephrine; FIGS. 2A-2B illustrate the appearance of Betta chromatophores prior to and after an exposure of a few hours duration to cholera toxin (CTX); FIGS. 3A-3B illustrate the appearance of a jewel cichlid scale (from Hemichromis bimaculatus) before and after, respectively, exposure to diisopropyl fluorophosphate (DFP); FIG. 3C illustrates eight scales from a jewel cichlid viewed in reflected light. Four scales were exposed to DFP and four scales were unexposed; FIG. 4A shows iridophore patches of a jewel cichlid scale before and after exposure to DFP; FIG. 4B is a graph of color changes of an iridophore patch as a function of time following exposure to DFP; FIG. 4C is a color-space rendering of a color trajectory of the iridophore patch of FIG. 4B following DFP exposure; FIG. 5A illustrates a segmentation of colors from iridophore patches before (top) and after (bottom) exposure to DFP; FIG. 5B is a graph of response of the iridophore patch of FIG. 5A as a function of time to various concentrations of DFP; FIGS. 6A-6C illustrate pigment aggregation in Betta chromatophores after exposure to norepinephrine (NE) without pre-incubation with CTX, with pre-incubation with a threshold concentration of CTX, and with pre-incubation with a substantial concentration of CTX, respectively; FIGS. 7A-11A illustrate the appearance of Betta splendens chromatophores prior to exposure and FIGS. 7B-11B illustrate the appearance of Betta splendens chromatophores after exposure, respectively, to various strains of bacteria; FIGS. 12A-12B illustrate the appearance of cultured chromatophores before and after, respectively, exposure to another strain of bacteria; FIG. 13 illustrates the appearance of a scale that includes several types of chromatophores after exposure to one or more analytes; FIGS. 14A-14D are photographs of chromatophores after exposure to bioactive agents; FIGS. 16A-16C are photographs of several Betta splendens chromatophore color variants unexposed to analytes; FIGS. 19A-19B are photographs of an erythrophore prior to and after exposure to NE in a solution containing calcium ions; FIGS. 20A-20B are photographs of an erythrophore prior to and after exposure to norepinephrine in a solution lacking

a person of ordinary skill in the art sufficient guidance to determine what the claims of the present application require in terms of the optical appearance of chromatophores prior to exposure to a bioactive compound or organism and subsequent to exposure.

Furthermore, Tables 1A and 1B (pages 42 and 44 of the present application, respectively) provide even further detail concerning chromatophore response to a bioactive compound or organism. Tables 1A and 1B provide numerous specific examples of bioactive compounds or organisms, and further recite the response of chromatophores exposed to a bioactive compound or organism. Based on this information alone, a person of ordinary skill in the art can determine what the claim language of all pending claims requires, and hence these claims are sufficiently definite to satisfy the requirements of 35 U.S.C. § 112, second paragraph.

Nevertheless, applicants have amended claim 7 to more specifically state "comparing an optical appearance of the first type of chromatophore and the second type of chromatophore prior to exposure to the bioactive compound or organism and after exposure to the bioactive compound". Similar language was found acceptable in Elving, U.S. Patent No. 4,985,353 (Elving), which has been cited against the present application. Since this language was acceptable in Elving, it also should be acceptable for the claims of the present application.

Applicants have amended claim 10 to recite "a sample potentially comprising a calcium channel blocker." Applicants also have amended claim 10 to recite producing an "erythrophore dispersion response" and "no melanophore response" to further clarify the optical change that is observed to indicate the presence of a calcium channel blocker. Support for this amendment can be found in the application, such as page 40.

Applicants have amended claim 11 to delete "color classes of chromatophores" and "in functional contact". Claim 11 now recites detecting a color change of one chromatophore from a first color to a second color. These amendments address the rejection under 35 U.S.C. § 112, second paragraph.

However, applicants believe that claim 11 as originally presented was sufficiently definite to satisfy the requirements of 35 U.S.C. § 112, second paragraph. "Color classes"

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calcium ions; FIG. 22 contains a graph of erythrophore response to verapmil; FIG. 23 contains graphs illustrating erythrophore response to BAPTA/AM and ionomycin; FIG. 24 illustrates erythrophore response to high and low concentrations of ryanodine.

means, as would be understood by a person of ordinary skill in the art, to refer to chromatophores having different colors, or shades, relative to one another, such as melanophores (black), erythrophores (red), and iridophores (yellow and/or iridescent). See page 8 of the application. "Functional contact" means an exposure sufficient to induce an observable change. Moreover, what does it matter how the color response is measured, such as simply by visual inspection, microscopy, spectroscopy, etc.? If the change is measurable by any such method, then the claim language is sufficiently definite.

The Office action states that "it is unclear how merely measuring a color response detects a bioactive compound." The chromatophores do not undergo a color change *sua sponte*. The color change is a direct result of a functional interaction with the bioactive compound or organism, and hence the color change is a direct indication that a bioactive compound or organism is present.

Applicants have canceled claim 12 without prejudice.

Applicants have amended claim 13 to address the antecedent basis problem noted by the Examiner.

Applicants have canceled claims 14-24 without prejudice.

Claim 25 refers to a test cell, and applicants believe that this term is understood by a person of ordinary skill in the art. However, solely to place the claims of the present application in condition for allowance, applicants have amended claim 25 to refer to bacteria.

Claim 26 has been canceled.

III. Prior Art Rejections

Claims 1-5, 11 and 25-27 are rejected as allegedly being anticipated by Elving, U.S. Patent No. 4,985,353 (Elving). Applicants traverse this rejection and request that it be withdrawn.

Applicants have amended claim 1 to recite "providing a capsule comprising chromatophores in a first optical state". Support for this feature is found throughout the application, such as at pages 16-25, and in Example 2.

Examiner Davis did not reject claim 12, which referred to encapsulating chromatophores, as being anticipated by Elving. Applicants agree that this is correct. Applicants find no mention of using capsules comprising chromatophores in Elving. Elving

simply teaches using solutions of pertussis toxin, or a body fluid sample, and solutions having fish scales, such as nutrient solution, and mixing such solutions in titer wells. Applicants' method as recited in claim 1 therefore is not anticipated by Elving, and applicants request that the rejection of claim 1 over Elving under 35 U.S.C. § 102(b) be withdrawn.

Claim 2 has been canceled without prejudice.

Claims 3-5 and 11 depend, either directly or indirectly, from claim 1, and are allowable for the reasons stated for claim 1, and further in view of the patentable combination of features recited in claim 1.

For example, claim 6 recites that the chromatophores are Betta chromatophores. Betta chromatophores are not taught by Elving.

Claim 7 recites using a first type and a second type of chromatophore, and claim 8 states that the first and second types of chromatophore are melanophores and erythrophores, respectively. Elving (as well as Lerner and Kotz) teaches using only one type of chromatophore to practice the disclosed method, and hence claims 7 and 8 are allowable for this additional reason. Moreover, improved sensitivity to bioactive compounds or organisms can be obtained using more than one type of chromatophore in combination. This result is discussed at various locations in the application, such as at page 12.

Claim 10 concerns identifying a calcium channel blocker. Elving does not teach or suggest such a method.

Claims 1-4, 11 and 25-27 are rejected under 35 U.S.C § 102(b) as allegedly being anticipated by Lerner *et al.* U.S. patent No. 5,462,856 (Lerner). Applicants traverse this rejection and request that it be withdrawn.

As discussed above, applicants have amended claim 1 to recite providing capsules comprising chromatophores. As with Elving, applicants find no disclosure in Lerner, nor suggestion, to provide capsules comprising chromatophores. Instead, all embodiments discussed in Lerner appear to teach using titer wells. As a result, claim 1 is not anticipated by Lerner.

Claim 2 has been canceled without prejudice.

Claims 3-4, 11 and 25-27 depend from claim 1, and hence are not anticipated by Lerner for the reasons stated above, and further in view of the patentable combination of features recited in these dependent claims.

Claims 1-9, 11 and 25-27 are rejected as allegedly being obvious under 35 U.S.C. § 103(a) as being unpatentable over Elving and/or Lerner. Applicants traverse this rejection and request that it be withdrawn.

As discussed above, applicants have amended claim 1 to recite using capsules comprising chromatophores. There is no teaching or suggestion in either Elving or Lerner that the chromatophores can be provided in capsules and further that the bioactive compound organism can be commingled with the chromatophores in the capsules to provide an observable response. Since neither Elving nor Lerner provides such teaching, the combination also can provide no such teaching or suggestion. Instead, both Elving and Lerner solely teach using titer plates, and mixing the required materials together in titer plates, not commingling materials in capsules. The cited references do not provide any process that teaches how to make capsules comprising chromatophores, nor do such references teach or suggest that beneficial results are obtained from using capsules comprising chromatophores.

In fact, the cited references implicitly suggest that the inventors of such processes never contemplated using capsules. For example, Elving teaches forming a first solution of specific antibodies, a second solution of pertussis toxin, a nutrient solution, and a fourth solution of a substance that induces a color change in the fish scale. These solutions are then all mixed together and incubated at 37°C. These method steps are not carried out in a sealed capsule.

Furthermore, prior to forming and testing encapsulated chromatophores, the present inventors had doubts that such formulations would work, but were pleasantly surprised that such formulations did work. The chromatophores live much longer than expected in sealed containers, and in fact live longer in a sealed environment than in unsealed formats. There is a general lore amongst cell biologists that subscribes to the fragility of living, primary cultures of cells (of which chromatophores are an example). Cell biologists generally would be surprised that the act of sealing the container (capsules, cartridges) is a favorable method that works as well as has been demonstrated for the present invention.

Finally capsules are more amenable to use in automated systems, such as biosensors. And, capsules are more easily transported than are solutions of materials. The present inventors have carried cartridges of chromatophores on coast-to-coast flights. Such cartridges are ready for use at any time (with an optical reader).

For the reasons stated above, claim 1 is not obvious in view of the combination of Elving and Lerner, and applicants request that the rejection under Section 103 be withdrawn.

Claim 2 is canceled.

Claims 3-9 depend from claim 1 and are allowable for the reasons stated above, and further in view of the patentable combinations of features recited in these claims.

For example, claim 5 recites particular types of organisms that can be detected using the claimed process. Neither Elving nor Lerner teach detecting organisms.

Claim 6 recites using Betta chromatophores. Elving teaches using certain fish chromatophores, but does not specifically teach Betta chromatophores. Lerner states that the pigment cells may be obtained generally from Pisces, such as *Zacco temmincki* (column 3, line 27), but does not identify Betta chromatophores.

Neither Elving nor Lerner appreciate that superior results can be obtained using Betta chromatophores. The present applicants have determined that Betta chromatophores are superior for use in detecting bioactive compounds or organisms relative to most other types of chromatophores. This is discussed in the application, such as at page 13-14. More specifically, the present application provides the following passages.

> Betta chromatophores are small in diameter in comparison with other fish chromatophores that are can be as large as 30 microns or more in effective diameter. In addition, Betta chromatophores tend to be uniform in size and can be densely packed to facilitate detection of color changes either visually or electronically. (See FIG. 15A.) Thus, dense, information-rich populations occupy small areas and can be interrogated with microscopic analysis and/or video recording.
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> As a specific example, 1,000 Betta splendens chromatophores can be situated in an area of less than 1 mm^2. In contrast, 1,000 Nile tilapia chromatophores require an area of about 100 mm^2. At higher densities, Nile tilapia chromatophores were observed to not thrive. Multispectral data collection is facilitated using Betta splendens chromatophores because uniform populations of colors are readily prepared. FIGS. 16A-16C illustrate the appearance of Betta splendens scales. FIG. 17 shows cultured Betta splendens chromatophores.
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> Betta chromatophores cultured in FSL Medium (described below) survived in a 24-well culture dish for 30 days. The chromatophores remained fully responsive to

norepinephrine (NE) during this time with cell numbers dropping by less than half at the end of 30 days. Most of this loss was attributable to the cell feeding method (the medium was suctioned away and the cells were then flooded with fresh medium). The remaining Betta chromatophores were healthy, responsive, and displayed normal morphologies. Any overgrowth of non-chromatophore cells such as epithelial cells and fibroblasts can be reduced or eliminated by differential centrifugation procedures that reduce these cell types during an initial plating of cultures.

In contrast, similarly prepared cultures of Nile tilapia melanophores were generally deteriorated at 30 days, with 90% of the cells either lost during medium changes, or remaining as non-responsive, morphologically abnormal remnants. The multispectral chromatophores of Hemichromis bimaculatus also exhibited substantial deterioration after less than 3 weeks in culture.

In addition, scales and fin slices from Betta splendens survive as active explants for at least 4 weeks, a time that is approximately as long as the longest survival time of chromatophores in explants from other tested fish species (Nile tilapia, Hemichromis bimaculatus, and zebrafish). Betta chromatophores can also survive exposure to broad temperature ranges. For example, Betta erythrophores were found to be tolerant of temperatures of up to 30°C for up to 1 week. Shorter exposure periods of 2 hours at temperatures up to 35°C did not affect viability. These upper temperature tolerance limits are a few degrees higher for Bettas than for chromatophores from Nile tilapia that generally cannot withstand 30°C temperatures for sustained periods.

Betta chromatophores are also relatively insensitive to changes in salinity and osmolarity. Betta chromatophores could be shifted into a FSL medium that was diluted by at least a 1:1 ratio with bacterial culture medium. Bacterial culture medium is different in both ionic composition and osmolarity from FSL. Similarly, tests of chemical agents have often entailed adding pure water as a diluent to FSL, and the ensuing decrease in ionic strength and osmolarity by at least 20% did not alter the responsiveness of the chromatophores in subsequent testing. Thus, Betta chromatophores can be effectively deployed in instrumentation and protocols that involve substantial changes in ionic strength and osmolarity.

Claim 7 recites using a first type of chromatophore, and a second type of chromatophore, and claim 8 recites that the first and second types of chromatophores are melanophores and erythrophore. Elving teaches that it is preferred to use solely melanophores from cuckoo wrasse, as such melanophores indicate presence of pertussis toxin that can be detected by visual inspection alone. Elving, column 2, line 66, through column 3, line 3. Lerner is primarily directed to using only one type of chromatophore from one species, particularly melanophores from *Xenopus laevis* and indicates that this is preferable as "melanophores from one source, such as ours, remains constant in terms of what receptors are expressed, [whereas] there can be stable genetic differences even within a single species."

Lerner, column, 11, lines 30-32. This, according to Lerner, can provide differential results. Therefore, Lerner effectively teaches away from using more than one type of chromatophore.

Claim 25 states that a bacteria is selected that produces a bacterial-induced response. Claim 26 is canceled. Both Elving and Lerner are very limited in their applications, and are solely concerned with particular compounds, not living cells. Elving is solely concerned with pertussis toxin, and provides no teaching or suggestion that cells can be exposed to chromatophores as recited in claim 25. Lerner is interested in identifying "**chemicals** that act as agonists or antagonists for proteins participating in signal transduction pathways that utilize heterotrimeric guanine nucleotide binding proteins . . . ." Emphasis added. Lerner, column 1, lines 17-20.

Claims 1-4, 6-11 and 25 are rejected under 35 U.C.S. § 103(a) as being unpatentable over Lerner in view of Kotz. Applicants traverse this rejection and request that it be withdrawn.

For the same reasons stated above, claim 1 is not anticipated, nor obvious, in view of Lerner. Kotz does not provide a teaching or suggestion of the features of applicants' pending claims that are not taught or suggested by Lerner. For example, applicants find no suggestion in Kotz to use capsules comprising chromatophores. As a result, claim 1 is not obvious in view of Lerner in combination with Kotz.

Moreover, the Office action states that it would have been obvious to use both melanophores and erythrophores together in a process. Applicants disagree. Lerner teaches using only one type of chromatophore from one species, particularly melanophores from *Xenopus laevis,* and indicates that this is preferable as melanophores from one source remain constant in terms of what receptors are expressed. Lerner, column, 11, lines 30-32. This, according to Lerner, can provide differential results. Therefore, Lerner effectively teaches away from using more than one type of chromatophore.

The Office action also states, in essence, that because there would be a reasonable expectation of success in using Betta chromatophores in view of the Lerner disclosure, that the use thereof as recited in certain of applicants' claims would be obvious. Again, applicants respectfully disagree for at least two reasons. First, obvious to try is an improper basis for concluding that a claimed invention is obvious. Second, Betta chromatophores provide a much

better result than would be expected from knowledge of a different chromatophore source as taught or suggested by Lerner and Kotz. Kotz teaches using chromatophores from squirrel fish. Hence Betta chromatophores are nonobvious in view of Lerner alone, or in combination with Kotz.

Claim 2 has been canceled.

Claims 3-4, 6-11 and 25 depend from claim 1, and are allowable for the reasons stated above, and further in view of the patentable combinations of features recited therein.

IV. New Claims

Applicants also have added new claims 28-39 to this application to recite additional combinations of features not taught or suggested by the references cited against this application.

For example, claims 28-30 further specify the structure of the capsules recited in claim 1. Because claim 1 is allowable in view of the cited references, claims 28-30 also are allowable.

Claims 31-32 specify particular materials used to make capsules, and these claims therefore are allowable for the reasons stated for claim 1.

Claim 33 recites exposing Betta chromatophores to the bioactive compound or organism. The cited references do not teach or suggest using Betta chromatophores, nor recognize the benefits that are associated with using such chromatophores relative to other types of chromatophores.

Claim 34 states that exposing comprises exposing two or more classes of Betta splendens chromatophores to the bioactive compound or organism. In addition to being allowable in view of specifying Betta splendens chromatophores, using two or more types of chromatophores provides superior bioactive compound or organism detection, which is not recognized by the cited references. Hence claim 34 is allowable for this additional reason.

New independent claim 37 recites quantifying a scalar optical change in at least one chromatophore in response to the bioactive compound. The method of claim 37 uses scalar (continuous) variables to objectively measure chromatophores. For example, *B. cereus* can be detected by measuring changes in pigment area, which is a scalar (continuous) physical value.

By contrast, the cited references use subjective categories to rate the appearance of chromatophores. For example, Elving used a categorical (ordinal) index to rank chromatophores on a subjective scale from 1 to 5, with 1 referring to the investigator's opinion of a chromatophore that is maximally aggregated, 2 being less aggregated than 1, 3 being less aggregated than 2, 4 being less aggregated than 3, and 5 being the least aggregated. Scalar methods do not rely on inherently inaccurate measurements made by eye. Also, scalar measurements enable powerful mathematical analyses and statistics, which allows accurate and correct quantities to be determined, such as the average perimeter of a population of erythrophores, or the mean value of the pigment area of a field of melanophores. It is a blatant error of statistical reasoning for categorical indices (such as that used by the cited references) to be averaged across a population of chromatophores. Such an approach is invalid just as it is invalid to speak of an average animal amongst several categories of animals: e.g., what is the average of three dogs, two cats and eight mice.

Finally, new claim 38 recites a combination of several features discussed above, each of which distinguishes the cited references by itself, including: (1) providing chromatophore capsules; (2) comprising two or more types of isolated, primary Betta splendens chromatophores; (3) introducing the chromatophores into the capsules to commingle the bioactive compound or organism with the chromatophores; and (d) detecting a scalar optical change in at least one chromatophore in response to the bioactive compound or organism.

The present application is in condition for allowance, and such action is requested.

Respectfully submitted,

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